Journal of Clinical Microbiology, June 2008, p. 1935–1940 Vol. 46, No. 6
0095-1137/08/$08.00+0 doi:10.1128/JCM.02403-07

Comparison of Sensitivities of Two Commercial Gamma Interferon Release Assays for Pulmonary Tuberculosis▽

Cynthia B. E. Chee,[1]* Suay H. Gan,[1] Kyi W. KhinMar,[1] Timothy M. Barkham,[2] Chwee K. Koh,[1] Shen Liang,[3] and Yee T. Wang[1]

Singapore Tuberculosis Control Unit, Tan Tock Seng Hospital, Singapore, Republic of Singapore[1]; Department of Pathology and Laboratory Medicine, Tan Tock Seng Hospital, Singapore, Republic of Singapore[2]; and Statistics Unit, Dean's Office, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, Republic of Singapore[3]

Received 16 December 2007/Returned for modification 25 January 2008/Accepted 31 March 2008

There are few head-to-head comparisons of the commercial gamma interferon release assays (GIRAs). We compared the performance of the T-SPOT.*TB* and QuantiFERON-TB Gold In-Tube (QFT-IT) assays in patients with culture-proven pulmonary tuberculosis. Blood was drawn for both assays within 14 days of starting antituberculosis treatment. The QFT-IT indeterminate rate was 3.5%; the T-SPOT.*TB* failure rate was 1.4%. There was poor agreement between the GIRAs ($\kappa = 0.257$) among the 270 patients with valid results for both tests. The sensitivities of the T-SPOT.*TB* and QFT-IT assays were 94.1 and 83.0%, respectively, with a significant difference in the performance of the assays (*P* = 0.001 [McNemar test]). Factors independently associated with indeterminate QFT-IT results were an age of ≥60 years (odds ratio [OR] 11.18, 95% confidence interval [CI] = 1.841 to 67.823, *P* = 0.009), female sex (OR = 7.47, 95% CI = 1.517 to 36.733, *P* = 0.013) and non-Chinese (i.e., Indian or Malay) race (OR = 7.89, 95% CI = 1.585 to 39.267, *P* = 0.012). The QFT-IT assay was significantly less sensitive in patients ≥60 years old (OR = 0.41, 95% CI = 0.181 to 0.918, *P* = 0.030) and in Indian compared to Chinese patients (OR = 0.27, 95% CI = 0.073 to 0.990, *P* = 0.048). The T-SPOT.*TB* assay was significantly less sensitive in Malay (OR = 0.23, 95% CI = 0.063 to 0.815, *P* = 0.023) and Indian patients (OR = 0.09, 95% CI = 0.017 to 0.429, *P* = 0.003) compared to Chinese patients. The performance of both assays was not significantly altered in diabetics. The diminished sensitivity of the GIRAs in persons of Malay and Indian race merits further study.

The commercial T-cell-based gamma interferon (IFN-γ) release assays (GIRAs) represent a long-awaited advancement in the field of tuberculosis (TB) diagnostics. These assays, which measure IFN-γ responses to the *Mycobacterium tuberculosis*-specific antigen early secretory antigenic target 6 (ESAT-6) and culture filtrate protein 10 (CFP-10), are widely anticipated to replace the century-old tuberculin skin test (TST) (4, 15, 23, 25, 27). The GIRAs are marketed as the T-SPOT.*TB* assay (Oxford Immunotec, Abingdon, United Kingdom) and the QuantiFERON-TB Gold (QFT-G) and QuantiFERON-TB Gold In-Tube (QFT-IT; Cellestis, Carnegie, Victoria, Australia) assays. The QFT-IT measures the IFN-γ response to Rv2654 (TB 7.7) antigen in addition to ESAT-6 and CFP-10.

Apart from their operational advantages over the TST, the GIRAs have demonstrated superior specificity (approaching 100%) over the TST in previously BCG-vaccinated persons (3, 14, 17, 22) and would be especially useful in overcoming the problem of false-positive TST responses due to cross-reactivity with *M. bovis* BCG vaccine. Regarding the sensitivity of the GIRAs, in the absence of a gold standard for latent TB infection (LTBI), most studies have utilized an exposure gradient to the infectious source case in contact investigations or active TB as surrogate markers for LTBI. Using exposure gradient, the T-SPOT.*TB* or its precursor versions have been shown to correlate better with exposure than the TST in point-source contact investigations in low-incidence settings (8, 16, 26, 30). Although it is recognized that active and latent TB states may manifest different T-cell-immune responses to the *M. tuberculosis*-specific antigens and that there is potential diminution of the cell-mediated immune response in active TB, many investigators have nonetheless utilized active TB as a surrogate for LTBI. A meta-analysis of studies using this surrogate showed a pooled sensitivity of 76% for the QFT-G and QFT-IT assays versus 88% for the enzyme-linked immunospot (ELISPOT) and T-SPOT.*TB* assay and 70% for TST using various cutoff readings (20).

To date, there have been few published head-to-head comparisons of the T-SPOT.*TB* and QFT-G/QFT-IT assays. Three such studies of active TB have reported a superior sensitivity of the T-SPOT.*TB* assay versus the QFT-G assay (9, 18, 11), while a study in pediatric patients showed equivalent sensitivities of the T-SPOT.*TB* and QFT-IT assays (7). A head-to-head study comparing the T-SPOT.*TB*, QFT-IT, and TST approaches in non-BCG-vaccinated subjects in a point-source contact investigation in The Netherlands showed both GIRAs to be significantly associated with hours of exposure (the QFT-IT assay more than the T-SPOT.*TB* assay), while the TST at cutoff of >15 mm did not show any association (1).

Although both the T-SPOT.*TB* and QFT-IT assays measure T-cell IFN-γ responses to similar *M. tuberculosis*-specific antigens over a 16- to 24-h incubation period, they are based on different technology platforms. The T-SPOT.*TB* assay is based on ELISPOT methodology and requires the isolation and incubation of peripheral blood mononuclear cells (PBMC) and

* Corresponding author. Mailing address: TB Control Unit, Dept. of Respiratory Medicine, Tan Tock Seng Hospital, 11, Jalan Tan Tock Seng, Singapore 308433, Republic of Singapore. Phone: 65 62566011. Fax: 65 63577871. E-mail: cynthia_chee@ttsh.com.sg.
▽ Published ahead of print on 9 April 2008.

the standardization of 250,000 PBMC in each of its test wells. The QFT-IT assay has technical and logistical advantages over the T-SPOT.*TB* assay, since the stimulation of T-cell IFN-γ response in whole blood is carried out in tubes precoated with the *M. tuberculosis* antigens. Although incubation followed by centrifugation are time-critical steps, IFN-γ detection, using an enzyme-linked immunosorbent assay, is time flexible and may be delayed for up to 4 weeks. However, since the background "noise" is higher, a "Nil" control is required in an attempt to adjust for this background, heterophile antibody effects, and nonspecific IFN-γ in blood samples. The T-SPOT.*TB* assay may be more laborious, but the use of a standardized number of washed PBMC presumably contributes to the greater sensitivity reported in the literature.

We compared the performance of the T-SPOT.*TB* and the QFT-IT assays in a cohort of culture-proven pulmonary TB (pTB) patients treated at the Singapore TB Control Unit (TBCU). We used baseline data collected as part of a larger study evaluating these assays for monitoring response to TB treatment and predicting relapse.

MATERIALS AND METHODS

This study was approved by the Domain Specific Institutional Review Board of the National Healthcare Group. The study population comprised patients evaluated and treated for pulmonary TB at the Singapore TBCU, the national referral center where ca. 60% of the country's cases are treated. The study subjects were prospectively recruited between April 2006 and May 2007. All participants gave informed consent.

Patients deemed likely to have pTB based on clinical and radiological findings were recruited within 2 weeks of starting TB treatment. At least two sputum specimens were obtained on separate days for acid-fast bacillus smears and TB culture and drug sensitivity testing prior to starting treatment. Peripheral venous blood was drawn for both GIRAs at the time of recruitment. TSTs were performed at the discretion of the attending physician. Human immunodeficiency virus (HIV) testing was routinely offered. Random blood glucose and liver enzymes were routinely performed prior to starting treatment. Since the main study required patients to be followed up for at least 2 years for relapse, those in whom the overall prognosis was guarded (e.g., the frail elderly or individuals with coexisting advanced malignancy) or those who did not reside permanently in Singapore were excluded from the study. Data on patient demographics, comorbidities, bacteriological status, and radiological findings were obtained. The treating physicians were blinded to the patients' GIRA results.

T-SPOT.*TB* assay. Blood was collected in dedicated tubes (BD Vacutainer, 10 ml, plus lithium heparin) and sent to the laboratory at room temperature within 6 h of sampling. The assay was performed and interpreted according to the manufacturer's instructions. The test result was considered positive if either or both panel A (containing ESAT-6 antigen) or panel B (containing CFP-10 antigen) had six or more spots than the negative control, and this number was at least twice the number of spots in the negative control. The test was considered failed if the negative control spot count was >10 or if there were <20 spots in the positive control, and both panels A and B were nonreactive according to the criteria above. Spots were counted with an ELISPOT reader (AID, Strasberg, Germany) and manually verified.

QFT-IT assay. Blood was collected in three heparinized 1-ml tubes provided as part of the kit; the "antigen" tube contained TB-specific stimulating antigens (ESAT-6, CFP-10, and TB7.7), the mitogen (positive control) tube contained phytohemagglutinin, and the third was a "nil" control tube. The assay was performed and interpreted according to the manufacturer's instructions.

Statistical analysis. Data obtained from questionnaires were entered and analyzed by using SPSS version 15. The outcomes of the two GIRAs (T-SPOT.*TB* and QFT-IT) were compared by using a McNemar test. A kappa test was used to evaluate the agreement between the two GIRAs.

The association between characteristics of subjects and indeterminate QFT-IT tests and positive T-SPOT.*TB* and QFT-IT results was evaluated by using the chi-square test or the Fisher exact test. Odds ratios (ORs) were estimated, and a logistical regression model was constructed to adjust for confounding factors and to obtain the adjusted ORs.

RESULTS

We recruited 350 participants treated for pTB at the TBCU from April 2006 to May 2007. *Mycobacterium tuberculosis* complex was isolated from the sputa of 286 (81.7%) patients. Nontuberculous mycobacteria (NTM) was isolated from the sputa of 13 patients, one of whom had dual growth (i.e., *M. tuberculosis* complex and NTM). Of the 51 sputum culture-negative patients, 38 were deemed active pTB on radiological and clinical grounds, and 13 patients were eventually deemed not to have pTB.

Characteristics of patients with sputum culture-positive pTB (n = 286). The median patient age for patients with sputum culture-positive pTB was 48.6 years (range, 17 to 77 years). There were 74 females (25.9%). The majority of the patients were Chinese (n = 195 [68.2%]), followed by Malaysian (n = 67 [23.4%]), Indian (n = 14 [4.9%]), and other races (n = 10 [3.5%]). Diabetes mellitus was the most common comorbidity, occurring in 103 patients (36.0%). Of the 238 subjects with known HIV status, seven were HIV positive. Two patients had end-stage renal failure, and one had malignant disease.

Thirteen patients had indeterminate or failed GIRA results: nine had indeterminate QFT-IT alone, one had indeterminate QFT-IT and failed T-SPOT.*TB*, and three had failed T-SPOT.*TB* results. Three patients were recruited after having received more than 14 days of TB treatment. All of these patients were excluded from the sensitivity analysis, which was performed on 270 culture-proven cases with positive or negative results for their T-SPOT.*TB* and QFT-IT tests taken within 14 days of starting TB treatment (Fig. 1). Of these, the vast majority (79%) were GIRA tested within 7 days.

Patients with indeterminate or failed GIRA results. The rate of indeterminate QFT-IT was 3.5% (10 of 286). All of the indeterminate results had mitogen minus nil values of <0.5 IU/ml and antigen minus nil values of <0.35 IU/ml. The T-SPOT.*TB* failure rate was 1.4% (4 of 286). Three failed results had positive control counts of <20 spot-forming cells (SFCs) and counts of ≤6 SFCs in both antigen wells, while one had a negative control count of >10 SFCs. Of the 10 patients with indeterminate QFT-IT results, one had failed T-SPOT.*TB*, one had negative T-SPOT.*TB*, and eight had positive T-SPOT.*TB* results. The patient with indeterminate QFT-IT and failed T-SPOT.*TB* results was a 75-year-old Malaysian diabetic man who was HIV negative. Seven of the ten patients with indeterminate QFT-IT and two of the four patients with failed T-SPOT.*TB* had diabetes.

Factors independently associated with indeterminate QFT-IT results were an age of ≥60 years (OR = 11.18, 95% confidence interval [CI] = 1.841 to 67.823, P = 0.009), being female (OR = 7.47, 95% CI = 1.517–36.733, P = 0.013), and belonging to a non-Chinese race (i.e., Indian or Malay) (OR = 7.89, 95% CI = 1.585 to 39.267, P = 0.012) (Table 1). Diabetes was significantly associated with indeterminate QFT-IT assay results on univariate analysis; however, this association was not found on multivariate analysis.

Comparison of T-SPOT.*TB* and QFT-IT assay results in 270 patients. The T-SPOT.*TB* assay result was positive in 254 subjects (94.1%), and the QFT-IT assay result was positive in 224 subjects (83.0%). There was a statistically significant difference

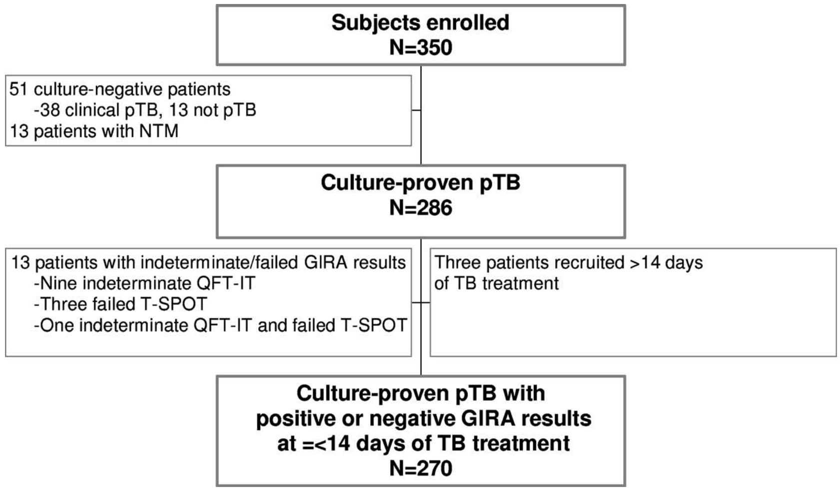

FIG. 1. There were 350 patients enrolled. The final analysis was performed on 270 sputum culture-positive subjects after exclusion of 51 culture-negative patients, 13 patients with NTM disease, 13 patients with indeterminate or failed GIRA results, and 3 patients who were recruited after having received more than 14 days of treatment.

in the performance of the two assays (P = 0.001 [McNemar test]) (Table 2). There was poor agreement between the two assays (kappa = 0.257). There was no significant difference in the sensitivity of the T-SPOT.*TB* assay between those tested within 7 days and those tested at 8 to 14 days of treatment (93.4% versus 96.5%, P = 0.536). Similarly, no difference was seen in the sensitivity of the QFT-IT assay between these two groups (83.1% versus 82.5%; P = 0.909). TSTs were performed for 217 patients. The TST sensitivities were 72.8% with ≥15 mm as the cutoff and 94.9% with ≥10 mm as the cutoff.

The T-SPOT.*TB* assay was significantly less likely to be positive in Malay (OR = 0.23, CI = 0.063 to 0.815, P = 0.023) and Indian (OR = 0.09, CI = 0.017 to 0.429, P = 0.003) patients compared to Chinese patients. Since all of the HIV-infected patients were T-SPOT.*TB* positive, analysis for this variable could not be performed (Table 3).

The QFT-IT assay result was significantly less likely to be positive in subjects ≥60 years of age (OR = 0.41, 95% CI = 0.181 to 0.918, P = 0.03) and in Indian patients compared to Chinese patients (OR = 0.27, 95% CI = 0.073 to 0.990, P = 0.048) (Table 4).

Quantitative GIRA results for Chinese versus non-Chinese patients. A comparison of the quantitative T-SPOT.*TB* results between Chinese and non-Chinese patients showed a statistically significant difference in the median number of SFCs above negative control in response to ESAT-6 (32.5 versus 17 SFCs/2.5 × 10^5 PBMC, P = 0.003) but not in response to CFP-10 (37.5 versus 34 SFCs/2.5 × 10^5 PBMC, P = 0.613).

TABLE 1. Univariate and multivariate analyses of patient characteristics associated with indeterminate QFT-IT assay results (10 of 280)[a]

Patient characteristics	Univariate analysis					Multivariate analysis		
	Total no. of tests performed	No. QFT indeterminate (%)	Crude OR	95% CI	P	Adjusted OR	95% CI	P
Age (yr)								
<60	227	6 (2.6)	1			1		
≥60	53	4 (7.5)	3.01	0.817–11.060	0.099	11.18	1.841–67.823	**0.009**
Gender								
Male	209	5 (2.4)	1			1		
Female	71	5 (7.0)	3.09	0.868–11.009	0.129	7.47	1.517–36.733	**0.013**
Ethnicity								
Chinese	189	3 (1.6)	1			1		
Non-Chinese	91	7 (7.7)	5.17	1.304–20.472	**0.015**	7.89	1.585–39.267	**0.012**
HIV status[b]								
Negative/unknown	273	10 (3.7)			1.00			
Positive	7	0 (0.0)						
Diabetes mellitus status								
Negative	180	3 (1.7)	1			1		
Positive	100	7 (7.0)	4.44	1.122–17.574	**0.038**	3.28	0.745–14.464	0.116

[a] Statistically significant P values are indicated in boldface.
[b] We were unable to estimate the risk since there were no indeterminate QFT results in the HIV-infected group.

TABLE 2. Numbers of positive and negative QFT-IT and T-SPOT.*TB* assay results

T-SPOT.*TB* results	No. of QFT-IT assay results		
	Negative	Positive	Total
Negative	10	6	16
Positive	36	218	254
Total	46	224	270[a]

[a] $P < 0.001$ (McNemar test).

There was no significant difference in the quantitative QFT-IT results of Chinese versus non-Chinese patients (median IFN-γ, 2.4 versus 1.8 IU/ml, $P = 0.521$).

Concordance or discordance in GIRA results. A total of 218 patients (80.7%) were T-SPOT.*TB* and QFT-IT positive; 36 (13.3%) were T-SPOT.*TB* positive and QFT-IT negative, 6 (2.2%) were T-SPOT.*TB* negative and QFT-IT positive, and 10 (3.7%) were both T-SPOT.*TB* and QFT-IT negative. The last group comprised a disproportionately high number of Malays (4 of 10) and Indians (4 of 10). The percentages of patients with dually negative tests among the various races were 6.3% for the Malay patients, 16.7% for the Indian patients, and 2.2% for the Chinese patients. There were no HIV-infected, renal failure, or cancer patients who tested negative with both T-SPOT.*TB* and QFT-IT assays.

DISCUSSION

A head-to-head comparison of the two commercial GIRAs showed poor agreement between the two assays and a significant difference in their performance in our cohort of 270 culture-proven pTB patients. The T-SPOT.*TB* was more sensitive than the QFT-IT (94.1% versus 83.0%). The sensitivity of the QFT-IT was significantly diminished in patients ≥60 years old. We found a disparity in the performance of the GIRAs in different races. Both assays were significantly less sensitive in Indians, with the T-SPOT.*TB* assay also less sensitive in Malays than in Chinese patients. Indeterminate QFT-IT results were more likely in persons ≥60 years old, females, and non-Chinese. The performance of the GIRAs was not significantly affected by the presence of diabetes.

It was not our aim to evaluate the clinical utility of the GIRAs in diagnosing or ruling out active TB. Rather, we took the opportunity to compare the performance of the two commercial GIRAs in culture-proven TB cases utilizing baseline data from a cohort of pTB patients recruited into a main study evaluating the utility of the GIRAs in monitoring treatment response and predicting relapse. Many of our subjects at enrollment were clinically obvious cases of active pTB in whom the use of the GIRAs as a diagnostic aid would not be necessarily indicated. Since the aim of the main study required successful treatment completion and a follow-up period of at least 2 years, we had excluded patients with advanced age, extreme frailty, and severe illness. Our study findings would thus not reflect the sensitivities of the GIRAs in such patients. Nevertheless, we believe that this comparative analysis still provides potentially useful information regarding the performance of the two commercial GIRAs.

To our knowledge, this is the largest cohort of culture-proven TB patients in whom a head-to-head comparison of the T-SPOT.*TB* and QFT-IT assays has been reported. The results showing the sensitivities of the T-SPOT.*TB* and QFT-IT tests for our patients with active TB confirm those reported in previous publications (6, 20, 28). The T-SPOT.*TB*'s consistently superior sensitivity is likely explained by the required

TABLE 3. Univariate and Multivariate analysis of patient characteristics associated with positive T-SPOT.*TB* assay results (254 of 270)[a]

Patient characteristics	Univariate analysis					Multivariate analysis		
	Total no. tested	No. T-SPOT positive (%)	Crude OR	95% CI	*P*	Adjusted OR	95% CI	*P*
Age (yr)		208 (94.1)						
<60	221	46 (93.9)	1		1.000			
≥60	49		0.96	0.262–3.500		0.56	0.132–2.423	0.442
Gender	204							
Male	66	191 (93.6)	1					
Female		63 (95.5)	1.43	0.395–5.178	0.77	1.59	0.400–6.303	0.511
Ethnicity								
Chinese	186	180 (96.8)	1					
Malay	63	57 (90.5)	0.32	0.098–1.020	0.08	0.23	0.063–0.815	**0.023**
Indian	12	9 (75.0)	0.10	0.21–0.466	**0.012**	0.09	0.017–0.429	**0.003**
Others	9	8 (88.9)	0.27	0.029–2.486	0.285	0.22	0.021–2.212	0.197
HIV status[b]								
Negative/unknown	263	247 (93.9)	-		1.00	-	-	-
Positive	7	7 (100.0)			-			
Diabetes mellitus status								
Negative	177	165 (93.2)	1					
Positive	93	89 (95.7)	1.62	0.507–5.165	0.412	2.24	0.650–7.756	0.201

[a] Statistically significant *P* values are indicated in boldface.
[b] We were unable to estimate the risk because the T-SPOT.*TB* assay was 100% positive in the HIV-infected group.

TABLE 4. Univariate and multivariate analyses of patient characteristics associated with positive QFT-IT assay results (224 of 270)[a]

Patient characteristics	Univariate analysis					Multivariate analysis		
	Total no. tested	No. QFT positive (%)	Crude OR	95% CI	*P*	Adjusted OR	95% CI	*P*
Age (yr)								
<60	221	188 (85.1)	1			1		
≥60	49	36 (73.5)	0.49	0.233–1.013	**0.051**	0.41	0.181–0.918	**0.030**
Gender	204	166 (81.4)	1			1		
Male	66	58 (87.9)	1.66	0.732–3.764	0.222	1.27	0.534–3.032	
Female								0.587
Ethnicity								
Chinese	186	159 (85.5)	1			1		
Malay	63	49 (77.8)	0.59	0.289–1.222	0.171	0.49	0.223–1.082	0.078
Indian	12	8 (66.7)	0.34	0.096–1.207	0.098	0.27	0.073–0.990	**0.048**
Others	9	8 (88.9)	1.36	0.163–11.301	1.000	0.89	0.103–7.674	0.914
HIV status								
Negative/unknown	263	220 (83.7)	1	0.056–1.206		1		
Positive	7	4 (57.1)	0.26		0.098	0.24	0.048–1.165	0.076
Diabetes mellitus status								
Negative	177	149 (84.2)	1	0.407–1.506		1		
Positive	93	75 (80.6)	0.78		0.463	0.92	0.454–1.847	0.805

[a] Statistically significant *P* values are indicated in boldface.

250,000 PBMC in each of its test wells, whereas the measurement of IFN-γ in the supernatant of whole blood in the QFT-IT test would adversely affect this assay's performance in immunosuppressed persons with low T-cell counts. Our relatively low QFT-IT indeterminate rate of 3.5% compared to that found in other studies (9, 10) may be due to our exclusion of frail elderly and severely ill patients. Despite this, we still found a higher indeterminate rate and diminished sensitivity of the QFT-IT in our older patients (i.e., those >60 years of age). The higher indeterminate QFT-IT rate in our female patients is unexplained. Although the sensitivity of the QFT-IT assay was not significantly diminished in our HIV-infected patients, there was a difference (which could be clinically important) in its performance compared to the T-SPOT.*TB* in these patients, the QFT-IT being positive in four of seven patients versus seven of seven patients for T-SPOT.*TB*. The 100% sensitivity of the T-SPOT.*TB* in our albeit small number of HIV-infected patients is consistent with the findings of other reports regarding this assay's undiminished sensitivity in patients with HIV infection or hematological malignancies using active TB or exposure as surrogates for LTBI (5, 19, 24). It may be argued that lowering the positivity threshold of the QFT-IT test should increase its sensitivity to that of the T-SPOT.*TB* assay. This will, however, be at the expense of reduced specificity. This issue could not be addressed in the present study since we did not seek to compare the performance of these assays in healthy controls.

The particular influences of racial and genetic factors on the performance of the commercial GIRAs have not been well studied in the clinical setting. It has been previously shown that the responses to ESAT-6 and CFP-10 varied between individuals with different HLA-DR types (2). A study in West African twins also reported that memory T-cell responses to "short-term culture filtrate" and peptides from the ESAT-6 protein are subject to genetic regulation (13). We showed here, for the first time, a disparity in the performance of the commercial GIRAs among different racial groups, with an increased likelihood of indeterminate QFT-IT results in Malays and Indians compared to Chinese patients, and diminished T-cell responses to the *M. tuberculosis*-specific antigens in Malays (with the T-SPOT.*TB* assay) and Indians (with both the T-SPOT.*TB* and QFT-IT assays) compared to Chinese. Another novel finding was the different quantitative T-cell responses to the individual antigens in the T-SPOT.*TB* assay, with the Chinese patients showing significantly greater quantitative responses to ESAT-6, but not to CFP-10, compared to the non-Chinese. The disparity in T-cell responses among these different races merits further study since this would have potential implications in the use and interpretation of the GIRAs among different populations, as well as in the field of TB vaccine research and development.

The performance of the GIRAs in patients with diabetes, an important TB risk factor, has not been widely reported. Diabetes is poised to be the "next epidemic"; the number of people with this condition worldwide is projected to increase from 171 million in 2000 to 366 million in 2030 (29). Southeast Asia and the Western Pacific region are at the forefront of the diabetes epidemic (12), and we anticipate that this situation would render TB control in these high-TB-burden regions an even greater challenge. Singapore has the highest incidence of diabetes in Asia, with a prevalence of 8.2%, which increases with age to 28.7% among those ≥60 years old (21). Although the presence of diabetes was not independently associated with diminished performance of either GIRA, a substantial proportion of patients with indeterminate QFT-IT or failed T-SPOT.*TB* results were diabetic. Whether the degree of diabetic control affected the T-cell response to the *M. tu-*

berculosis-specific antigens was not specifically addressed by our study design.

In conclusion, head-to-head comparison of the T-SPOT.*TB* and QFT-IT assay results in 270 culture-positive pTB patients demonstrated a superior sensitivity of the T-SPOT.*TB* over the QFT-IT using the manufacturers' cutoffs. The performance of the QFT-IT assay was diminished in patients older than 60 years. Our finding of decreased sensitivity of the GIRAs in patients of Indian or Malay race (compared to Chinese) highlights the need for further studies pertaining to the use and interpretation of these assays in different racial groups.

ACKNOWLEDGMENTS

We thank all of the patients who participated in the study. We also thank laboratory technician Agampodi Pereira, Nursing Office Kwee-Yin Han, and all medical and nursing staff of the TBCU.

This study was supported by the National Medical Research Council grant 1004 / 2005 from the Singapore Ministry of Health.

REFERENCES

1. **Arend, S. M., S. F. Thijsen, E. M. Leyten, J. J. Bouwman, W. P. Franken, B. F. Koster, F. G. Cobelens, A.-J. van Houte, and A. W. Bossink.** 2007. Comparison of two interferon-gamma assays and the tuberculin skin test for tracing tuberculosis contacts. Am. J. Respir. Crit. Care Med. **175:**618–627.
2. **Arend, S. M., A. Geluk, K. F. van Meigaarden, J. T. van Dissel, M. Thiesen, P. Andersen, and T. H. Ottenhoff.** 2000. Antigenic equivalence of human T-cell responses to *Mycobacterium tuberculosis*-specific RD 1-encoded protein antigens ESAT-6 and culture filtrate protein 10 and to mixtures of synthetic peptides. Infect. Immun. **68:**3314–3321.
3. **Brock, I., K. Weldingh, T. Lillebaek, F. Follmann, and P. Andersen.** 2004. Comparison of tuberculin skin test and new specific blood test in tuberculosis contacts. Am. J. Respir. Crit. Care Med. **170:**65–69.
4. **Centers for Disease Control and Prevention.** 2005. Guidelines for using the QuantiFERON-TB Gold test for detecting *Mycobacterium tuberculosis* infection. United States. MMWR Morb. Mortal. Wkly. Rep. **54:**49–56.
5. **Chapman, A. L., M. Munkanta, K. A. Wilkinson, A. A. Pathan, K. Ewer, H. Ayles, W. H. Reece, A. Mwinga, P. Godfrey-Faussett, and A. Lalvani.** 2002. Rapid detection of active and latent tuberculosis infection in HIV-positive individuals by enumeration of *Mycobacterium tuberculosis*-specific T cells. AIDS **16:**2285–2293.
6. **Dewan, P. K., J. Grinsdale, and L. M. Kawamura.** 2007. Low sensitivity of a whole-blood interferon-γ release assay for detection of active tuberculosis. Clin. Infect. Dis. **44:**69–73.
7. **Detjen, A. K., T. Keil, S. Roll, B. Hauer, H. Mauch, U. Wahn, and K. Magdorf.** 2007. Interferon-gamma release assays improve the diagnosis of tuberculosis and nontuberculous mycobacterial disease in children in a country with a low incidence of tuberculosis. Clin. Infect. Dis. **45:**322–328.
8. **Ewer, K., J. Deeks, L. Alvarez, G. Byrant, S. Walker, P. Andersen, P. Monk, and A. Lalvani.** 2003. Comparison of T-cell-based assay with tuberculin skin test for diagnosis of *Mycobacterium tuberculosis* infection in a school tuberculosis outbreak. Lancet **361:**1168–1173.
9. **Ferrara, G., M. Losi, R. D'Amico, P. Roversi, R. Piro, M. Meacci, B. Meccugni, I. M. Dori, A. Andreani, B. M. Bergamini, C. Mussini, F. Rumpianesi, L. M. Fabbri, and L. Richeldi.** 2006. Use in routine clinical practice of two commercial blood tests for diagnosis of infection with *Mycobacterium tuberculosis*: a prospective study. Lancet **367:**1328–1334.
10. **Ferrara, G., M. Losi, M. Meacci, B. Meccugni, R. Piro, P. Roversi, B. M. Bergamini, R. D'Amico, P. Marchegiano, F. Rumpianesi, L. M. Fabbri, and L. Richeldi.** 2005. Routine hospital use of a commercial whole blood interferon-γ assay for tuberculosis infection. Am. J. Respir. Crit. Care Med. **172:**631–635.
11. **Goletti, D., S. Carrara, D. Vincenti, C. Saltini, E. Busi-Rizzi, V. Schinina, G. Ippolito, M. Amicosante, and E. Girardi.** 2006. Accuracy of an immune diagnostic assay based on RD1 selected epitopes for active tuberculosis in a clinical setting: a pilot study. Clin. Microbiol. Infect. **12:**544–550.
12. **Hossain, P., B. Kawar, and M. El Nahas.** 2007. Obesity and diabetes in the developing world: a growing challenge. N. Engl. J. Med. **356:**213–215.
13. **Jepson, A., A. Fowler, W. Banya, M. Singh, S. Bennett, H. Whittle, and A. V. Hill.** 2001. Genetic regulation of acquired immune responses to antigens of *Mycobacterium tuberculosis*: a study of twins in West Africa. Infect. Immun. **69:**3989–3994.
14. **Kang, Y. A., H. W. Lee, H. I. Yoon, B. Cho, S. K. Han, Y. S. Shim, and J. J. Yim.** 2005. Discrepancy between the tuberculin skin test and the whole-blood interferon gamma assay for the diagnosis of latent tuberculosis infection in an intermediate tuberculosis-burden country. JAMA **293:**2756–2761.
15. **Lalvani, A.** 2007. Diagnosing tuberculosis infection in the 21st century. New tools to tackle an old enemy. Chest **131:**1898–1906.
16. **Lalvani, A., A. A. Pathan, H. Durkan, K. A. Wilkinson, A. Whelan, J. J. Deeks, W. H. H. Reece, M. Latif, G. Pasvol, and A. V. S. Hill.** 2001. Enhanced contact tracing and spatial tracking of *Mycobacterium tuberculosis* infection by enumeration of antigen-specific T cells. Lancet **357:**2017–2021.
17. **Lalvani, A., A. A. Pathan, H. McShane, R. J. Wilkinson, M. Latif, C. P. Conlon, G. Pasvol, and A. V. S. Hill.** 2001. Rapid detection of *Mycobacterium tuberculosis* infection by enumeration of antigen-specific T cells. Am. J. Respir. Crit. Care Med. **163:**824–828.
18. **Lee, J. Y., H. J. Choi, I.-N. Park, S.-B. Hong, Y.-M. Oh, C.-M. Lim, S. D. Lee, Y. Koh, W. S. Kim, D. S. Kim, W. D. Kim, and T. S. Shim.** 2006. Comparison of two commercial interferon-γ assays for diagnosing *Mycobacterium tuberculosis* infection. Eur. Respir. J. **28:**24–30.
19. **Liebeschuetz, S., S. Bamber, K. Ewer, J. Deeks, A. A. Pathan, and A. Lalvani.** 2004. Diagnosis of tuberculosis in South African children with a T-cell-based assay: a prospective cohort study. Lancet **364:**2196–2203.
20. **Menzies, D., M. Pai, and G. Comstock.** 2007. Meta-analysis: new tests for the diagnosis of latent tuberculosis infection: areas of uncertainty and recommendations for research. Ann. Intern. Med. **146:**340–354.
21. **Ministry of Health, Singapore.** 2004. Report of the National Health Survey 2004. Ministry of Health, Singapore, Republic of Singapore.
22. **Mori, T., M. Sakatani, F. Yamagushi, T. Takashima, Y. Kawabe, K. Nagao, E. Shigeto, N. Harada, S. Mitarai, M. Okada, K. Suzuki, Y. Inoue, K. Tsuyuguchi, Y. Sasaki, G. H. Mazurek, and I. Tsuyguchi.** 2004. Specific detection of tuberculosis infection: an interferon-gamma-based assay using new antigens. Am. J. Respir. Crit. Care Med. **170:**59–64.
23. **Pai, M., S. Kalantri, and K. Dheda.** 2006. New tools and emerging technologies for the diagnosis of tuberculosis. I. Latent tuberculosis. Expert Rev. Mol. Diagn. **6:**413–422.
24. **Piana, F., L. R. Codecasa, P. Cavallerio, M. Ferrarese, G. B. Migliori, L. Barbaraso, E. Morra, and D. M. Cirillo.** 2006. Use of a T-cell-based test for detection of tuberculosis infection among immunocompromised patients. Eur. Respir. J. **28:**31–34.
25. **Richeldi, L.** 2006. An update on the diagnosis of tuberculosis infection. Am. J. Respir. Crit. Care Med. **174:**736–742.
26. **Richeldi, L., K. Ewer, M. Losi, B. M. Bergamini, P. Roversi, J. Deeks, L. M. Fabbri, and A. Lalvani.** 2004. T cell-based tracking of multidrug resistant tuberculosis after brief exposure. Am. J. Respir. Crit. Care Med. **170:**288–295.
27. **Rothel, J. S., and P. Andersen.** 2005. Diagnosis of latent *Mycobacterium tuberculosis* infection: is the demise of the Mantoux test imminent? Expert Rev. Anti-Infect. Ther. **3:**981–993.
28. **Tsiouris, S. J., D. Coetzee, P. L. Toro, J. Austin, Z. Stein, and E.-S. Wafa.** 2006. Sensitivity analysis and potential uses of a novel gamma interferon release assay for diagnosis of tuberculosis. J. Clin. Microbiol. **44:**2844–2850.
29. **Wild, S., G. Roglic, A. Green, R. Sicree, and H. King.** 2004. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care **27:**217–223.
30. **Zellweger, J. P., A. Zellweger, S. Ansermet, B. de Senarclens, and P. Wrighton-Smith.** 2005. Contact tracing using a new T-cell-based test: better correlation with tuberculosis exposure than the tuberculin skin test. Int. J. Tuberc. Lung Dis. **9:**1242–1247.